RESEARCH ARTICLE

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Increased Expression of the *ABCA1* and *ABCA3* Transporter Genes is Associated with Cisplatin Resistance in Breast Cancer Cells

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Abstract

Objective: Breast cancer (BC) is a highly malignant neoplasm with resistance to therapeutics that are related to genes associated with multidrug resistance. The excessive expression of ATP-binding cassette transporters (ABCs) genes, including *ABCA1* and *ABCA3*, is a primary factor contributing to the increased effluent of cell-toxic drugs and subsequent treatment resistance. Therefore, the current work aimed to explore the role of *ABCA1* and *ABCA3* in chemoresistance activity against cisplatin in breast cancer cells. **Methods:** The current study compared the AMJ13 breast cancer cells derived from a woman Iraqi patient, which are hormone receptor-negative, with MCF-7 breast cancer cells, which are hormone receptor-positive. Cytotoxic assay (CCK-8 assay) is used to measure the cell's viability and cytotoxic activity after it has been treated with cisplatin. Morphological Study using crystal violet stain to examine cytological changes was conducted. Quantitative RT-PCR is used to measure how much the *ABCA1*, and 3 genes mRNA are being expressed before and after treatment. **Results:** The CCK-8 assay found that IC_{50} values of cisplatin in AMJ13 and MCF-7 cells were 202.2 µg/ml and 90.23 µg/ml, respectively. The IC_{50} value of AMJ13 is 2-fold higher than in MCF-7 cells. The QPCR study revealed that breast cancer cell lines AMJ13 and MCF-7 subjected to cisplatin showed upregulated levels of *ABCA1* and *ABCA3* expression. Experiments with cytotoxicity assays demonstrate that higher expression of *ABCA1* and *ABCA3* in AMJ13 and MCF-7 breast cancer cell lines is linked to their resistance. **Conclusion:** The findings of this study suggest that the *ABCA1* and *ABCA3* transporters play a significant role in the resistance to cisplatin and, therefore, may represent a potential therapeutic target to assist patients with BC in overcoming the resistance to cisplatin.

Keywords: Breast cancer- ABCA transporters- Drug resistance- Cisplatin- chemotherapeutics- ATP-Binding cassette

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Introduction

Breast cancer (BC) constitutes the most prevalent neoplastic, is frequently detected, and is the primary contributor to cancer-associated fatalities among women globally. There were an estimated 2.3 million new cases of BC reported, resulting in a mortality rate of around 685 thousand related to this clinical disease through 2020. This increase in incidence may be attributed to the growth and age of the global population, as well as the adoption of lifestyles that promote cancer (Moslemi et al., 2021; Sung et al., 2021; Rasheed, 2022). Iraqi females also showed a high percentage of new cases according to WHO studies (Abdulla et al., 2022).

Cisplatin, cis-diamminedichloroplatinum (II) (CDDP) is a highly effective antineoplastic drug derived from platinum that is extensively utilized in the therapeutic management of different types of carcinomas and sarcomas (Pooja et al., 2018). The primary mechanism underlying the effectiveness of this drug is attributable to its ability to induce crosslinking between DNA purine bases, consequently disrupting the DNA repair pathways in cancer cells. This interference ultimately results in DNA damage and cell apoptosis via caspase-3 activation and X-linked inhibitor of apoptosis (XIAP) expression (Romani, 2022). Furthermore, tumor cells can develop resistance to the damages that are induced by cisplatin by undergoing genetic and epigenetic alterations; these abnormalities lead to the triggering of intrinsically resistant mechanisms within cancer cells (Lugones et al., 2022).

Drug transport is just one of several cellular processes that are mediated by a set of complicated mechanisms responsible for the passive and active transport of various substances. Ion channels, ATP pumps, transporters, and aquaporins among other proteins, can be identified as

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they cross the membrane. The most well-known group of membrane transport proteins is the ATP-binding cassette (ABC) superfamily (Kulbacka et al., 2017; Hassanpour et al., 2018). In humans, there are 49 ABC transporter subtypes, including a pseudogene. Based on their gene structure, amino acid sequences, domain arrangement, and phylogenetic study, these 49 ABCs are further subdivided into 7 subfamilies, ABCA through ABCG (Locher, 2016; Dvorak et al., 2017). The ABCA subfamily is comprised of 13 complete transporter members and has been classified into two subgroups; the first (*ABCA5*, A6, and A8-10) is encoded by a cluster of genes localized on chromosome 17q24.2-q24.3, and their amino acid sequences share a similarity of 53–78%. While, the second subgroup includes (*ABCA1*-4, A7, and A11-13, which are encoded by genes distributed on six different chromosomes, the human *ABCA11* gene was initially misidentified as nonfunctional (Albrecht and Viturro, 2007; Molday, 2015). Structurally, the complete ABCA transporter has two transmembrane domains (TMDs) and two nucleotide-binding domains (NBDs). Each TMD is made up of six transmembrane spans. During substrate transport, conformational alterations in the extracellular domains of TMDs can occur as a result of the ATP hydrolysis of NBDs (Taylor et al., 2017).

The majority of ABC transporters related to this subfamily are involved in regulating membrane trafficking and function, as well as lipids transporting and homeostasis as shone in Table 1, (Borst et al., 2000). *ABCA1* protein is involved with the transportation of phospholipids into the plasmic membranes and also in the reversed transfer of cholesterol from cells into HDLs (high-density lipoproteins) inside the bloodstream. Similar to this, a high level of cholesterol influx through LDLs (low-density lipoproteins) encourages the upregulation of *ABCA2*, A3, and A7 transporters genes to become more active. *ABCA3* protein is essential for facilitating the transportation of mainly saturated phosphatidylcholine to the membranes of lung surfactant, which in turn fills the lamellar cell/bodies located within type-2 cells of the lung epithelium (Yamano et al., 2001). This indicates that these transfer proteins have a significant impact in preserving cholesterol levels on the cellular environment at a healthy level (Oram and Vaughan, 2000; Davis Jr, 2011).

ABC transporters have a strong impact on cancer, and they are closely associated with their ability to efflux drugs or toxins, as well as various signaling molecules such as cholesterol and other lipids. These proteins have the potential to influence the biological functions that regulate the proliferating, surviving, and migrating of cancer cells through several mechanisms operating at multiple stages (Domenichini et al., 2019; Nobili et al., 2020). There have been reports of ABCA subfamily transporter upregulation in tumor development, poor prognosis, and anticancer drug resistance (Modi et al., 2022). However, ABCA transporters show a complicated and controversial role in cancer. Generally, in vitro, research has established a link between the emergence of chemotherapy resistance and multiple members of the ABCA subfamily (Domenichini et al., 2019). *ABCA1* overexpression was correlated with a worsened prediction for colorectal cancer via mediating an unbalanced intracellular level of cholesterol (Alketbi et al., 2023). Studies have also demonstrated that HDL can increase the proliferative and migratory abilities of prostate cancer cells by triggering the ERK1/2 signal route, which is facilitated by *ABCA1* (Sekine et al., 2010; Aguirre-Portolés et al., 2018). Regarding other ABCA subfamily members, the overexpression of *ABCA2* appears to be accountable for multidrug resistance (MDR) in certain kinds of cancer, including EDC (Estrogen-dependent cancers) and SCLC (Small Cell Lung Cancers), due to its function in drug efflux. (Mack et al., 2012; Gao et al., 2015). Furthermore, according to studying using clinical samples of infant acute lymphoblastic leukemia (ALL) revealed that co-overexpression of *ABCA2* and *ABCA3* together may potentially be correlated to a bad prognosis and drug resistance with many cases of ALL (Rahgozar et al., 2014).

Numerous studies conducted in a clinical context suggested a correlation between the expression of ABCA transporters and poor responses to anti-cancer drugs. Patients diagnosed with ovarian cancer who exhibit significantly elevated expression levels of *ABCA1*, A6, A8, and A9 genes have a considerably lower chance of survival. (Hedditch et al., 2014; Elsnerova et al., 2017). Another investigation showed that amount of *ABCA3* expression represented a distinct risk for determining cancer survival or reoccurring (Schimanski et al., 2009). Accumulated clinical evidence reveals a correlation between the upregulation of ABCA transporters and bad outcomes in various types of malignancies, including prostate, stomach, kidney, and leukemias (Araújo et al., 2016; Yun et al., 2017).

The current study compared the AMJ13 breast cancer cells derived from a woman Iraqi patient, which are hormone receptor-negative, with MCF-7 breast cancer cells, which are hormone receptor-positive, in regard to resistance to cisplatin and the association of this resistance with the expression of intracellular ABC transporter genes *ABCA1* and *ABCA3*, which may confer multidrug resistance in BC cells. The study results may suggest that *ABCA1* and *ABCA3* transporter proteins have promise as therapeutic targets in individual management for patients with BC, regardless of the presence or absence of hormone receptor expression.

Materials and Methods

Cell Culture

Two distinct lines of cells associated with breast cancer, namely AMJ13 and MCF-7, were used in this study. Cells obtained from a patient of Iraq were used to establish AMJ13 cell lines of breast cancer. The main tumor of a woman patient from Iraq, age 70, was confirmed to be infiltrated by ductal carcinomas during histological examination. The results obtained from immunocytochemistry analysis indicated the absence of expression for both the estrogen and progesterone receptors. However, a slightly positive outcome was reported with regard to *HER2/neu* gene expression. Their passage number was 2, this cell line considered as a hormone therapy nonresponsive cell line (Al-Shammari

et al., 2015). While cells of MCF-7 is characterized by its positive expression for both estrogen and progesterone receptors, making it a valuable model for studying breast cancer. (Soule et al., 1973). This cell line was reported to be resistant to cisplatin (Kashkoulinejad-Kouhi et al., 2021). Tissue culture flasks (T25 cm2; Falcon/United States) were used for cultivating the cells in RPMI 1640 medium (Capricorn/Germany), complemented by 10 percent serum of fetal bovine (Capricorn/Germany), 100 units/ml of penicillin, and 100 µg/ml of streptomycin (Sun et al., 2019; Mohammed et al., 2022). Subculturing of cell lines occurred when the monolayers reached confluence. After removing the cell sheets from the growing medium, it was rinsed twice using a 2 ml solution of trypsin EDTA. After that, the monolayer cell was treated with 1 ml of trypsin again, and then the flask was carefully shaken. A few minutes were spent incubating the culture cells at 37°C until they detach; trypsinization was stopped by adding a growth medium; and then the cells were distributed at the desired concentration. Following this, the cultured flasks were re-incubated at a temperature of 37°C (Al-Ziaydi et al., 2020). The cell lines provided by the cell bank unite were authenticated regularly as standard work by the supplier.

Cisplatin Cytotoxicity

The WST-8/CCK-8 (Enhanced Cell Counting Kit-8) assay (Santa Cruz Biotechnology/United States) was performed by 96 well-flatted bottoms to measure the cytotoxic effects of cisplatin. After 24 hours, a confluent monolayer developed when AMJ13 and MCF-7 cells seeded at an average number of 7,000 cells per well. The experiment was done in triplicates. Multiple doses of cisplatin (1000, 500, 250, 125, 62.5, and 31.2 µg/ml) were used to treat cancer cells, and the assessment of cell viability was conducted by eliminating the media following a 72-hour exposure period. Two hours later, in an incubator at 37°C, a volume of 50 ml of solution from the WST-8/CC kit-8 (Elabscience/China) was added to the cells (Kano et al., 2017). The absorbance was quantified at a wavelength of 450 nm by employing a microplate reader, and the procedure was conducted in triple. The proportion for cytotoxicity, or the incidence at which cell proliferation was stopped, was computed using the next formula. When A refers to the OD (optical density) for control while B refers to the OD for samples, the inhibition rate is calculated as A - B / A*100 (Singh et al., 2018; Abd-Elhady et al., 2021).

Morphological Study

Both the treated and untreated cancer cells were stained by adding 100 µl of crystal violet dye per well and incubating the plate at temperature of 37 °C for 20 minutes. Following this, the microtiter plate was washed thoroughly. This experiment is designed to examine the morphological changes induced by cisplatin.

Extracted RNA and performed Real-Time PCR for Quantitative Analysis

Breast cancer cell lines AMJ13 and MCF-7 were incubated for 24 hours before being treated with cisplatin at concentrations of 202.2 µg/ml and 90.23 µg/ml, respectively, which represent their IC_{50} values. After that, 1X PBS (phosphate buffer saline) was used to wash the cells once. Following this, 1 ml of TRIzol (Thermo Fisher Scientific/United States) was used for the purpose of extracting total RNA from the cellular samples. The quality of the samples for downstream applications was determined by measuring the amount and stability of RNA extracted utilizing a Quantus Fluorometer (Abo-Altemen et al., 2019).

The qRT-PCR (Quantitative Real-Time Polymerase Chain Reaction) was conducted applying the GoTaq® 1-Step of RT-qPCR System. The ACTB (Beta-actin) gene, which was supplied by Integrated DNA Technologies, USA (IDT), was utilized as a standard of internal control for normalization the work. The relative formula of the quantified approach (2^-ΔΔCt) was used to figure out the fold change (Livak and Schmittgen, 2001). The following primers were used: *ABCA1*: Forward- 5'- CAGCAGTTGGATGGCTTAGA-3', Revers- 5'- CCAGGTGTACACAGAACCATTA-3', and *ABCA3*: Forward- 5'- GAGAAGGAAAGGAGGCTGAAG-3', Revers- 5'- GGAGGAAGAGGAAGAACAAGAG-3'.

These primers have been designed by Primer Quest™ Tool and manufactured by Integrated DNA Technologies (IDT) USA. The thermal cycler program is illustrated in Table 2, below:

The experiment of the RT-PCR reaction for gene expression has been repeated in a triplicate, which was adopted with all genes in this study, to achieve the best values (Al-Shammari et al., 2021).

Statistical Analysis

The obtained data from the cytotoxicity assay were statically analyzed and calculated the IC50 value for the cisplatin on each cell line was used, using an analysis of variance (ANOVA) multiple comparison test by Graph Pad Prism-9 (Graph Pad Software Inc., SanDiego/United States). These values are displayed as the mean ± SD for measures performed in triplicate.

Results

In Vitro, AMJ13 and MCF-7 Cell lines Displayed Resistance to Cisplatin

In this experiment, cell lines AMJ13 and MCF7 were exposed to cisplatin over a period of 72 hours at varying doses. The CCK-8 assay found that the half maximum of IC_{50} (Inhibitory Concentrations) values of cisplatin in AMJ13 and MCF-7 cells were 202.2 µg/ml and 90.23 µg/ml, respectively. The IC_{50} value of AMJ13 exhibited a greater than 2-fold increase in comparison to MCF-7 cells as shown in Figure 1A. This confirmed that AMJ13 and MCF-7 are resistant to chemotherapeutic agents, especially cisplatin. The high IC_{50} doses exhibited mild lesions and damage in the cells as illustrated in Figure 1B, where the cells stained with crystal violet stain.

ABCA1 and ABCA3 Genes Overexpression Promote Cisplatin Resistance in AMJ13 and MCF-7 Cells

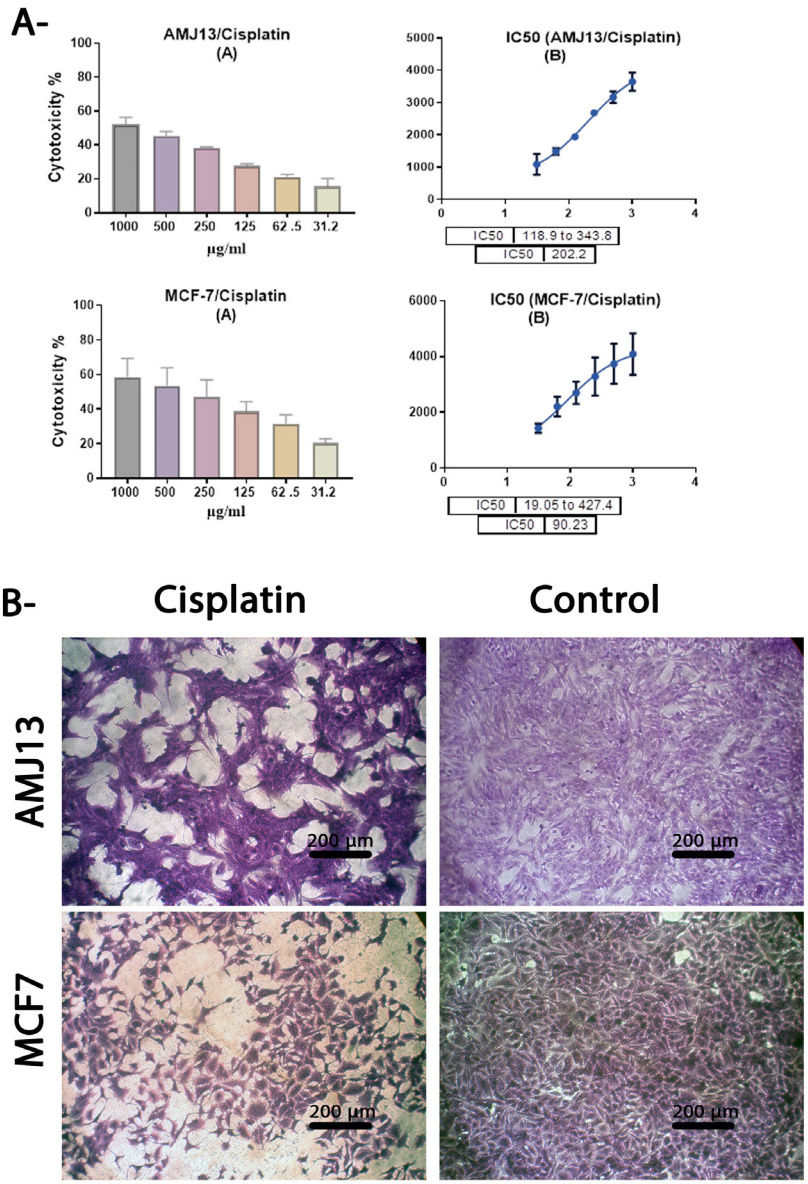

Figure 1. (A) Cisplatin cytotoxicity histogram in AMJ13 and MCF-7 cells (72 hours of incubation). It also displayed the IC50 value of the cisplatin dose. (B) Breast cancer cells (AMJ13 and MCF-7) under an inverted microscope after 72h of untreated and treated with cisplatin, after crystal violet staining, which shows the presence of cell detachment and the remaining viable cells of large size and with fewer cells showing mild lesions such as cell rounding and nuclear condensation. Size bar: 200µm.

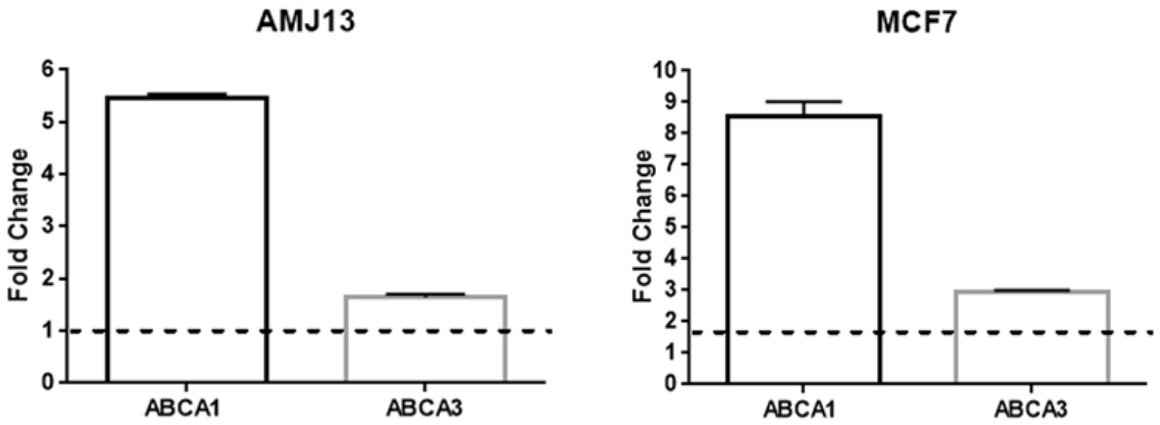

Figure 2. High Expression of *ABCA1* and *ABCA3* Genes Increases Resistance of AMJ13 and MCF-7 Cells to Cisplatin.

Table 1. The Characteristic Features and Physiological Roles of *ABCA* Transporter Subfamily Genes

Genes	-Cellular location -Tissue expression	Substrates	Physiological function and interpretation	Reference
ABCA1	-Plasma membranes and endoplasmic reticulum -Lung, liver, nerve system, colon, testis	Cholesterol, Phospholipids, Cisplatin, Statins, Doxorubicin	Transport Cholesterol to HDL pathway, Lipid exchange, Activating ERK1/2 rout to promote cell proliferation	(Du et al., 2015; Wang et al., 2021)
ABCA2	-Plasma membranes and lysosomal membranes -Nervous system	Lipids, Mitoxantrone, Estramustine	Phospholipid transport, Mediates multidrug resistance (MDR) in SCLC	(Vedhachalam et al., 2007; Davis Jr, 2014)
ABCA3	Lysosomal membranes, plasmatic membranes, lamellar/cell bodies and extracellular regions -Lung	Cholesterol, Anthracycline, Imatinib	Lipid constituents of the surfactant-based pulmonary are transported, Regulating of cholesterol, Weak chemotherapy responses in cancer, and a risk indicator of recurrence or survival in BC	(Aberuyi et al., 2017; Wang et al., 2021)
ABCA4	-Plasma membranes -Photoreceptors	N-retinylidene-PE, Vitamin A	In photoreceptors, phosphatidylethanolamines and N-retinylidene-phosphatidylethanolamines (PE) are moved across the membrane along the luminal side to the cytoplasm	(Quazi et al., 2012; Scortecci et al., 2021)
ABCA5	-Lysosomal membranes, endosomal membranes -Muscle, heart, testis	Lysosome, Cholesterol, Tacrolimus	Regulator of intracellular lysosomal movement, a urinary biomarker for detection of PIN (Prostatic Intraepithelial Neoplasia).	(Fu et al., 2015; Sequeiros et al., 2015)
ABCA6	-Plasma membrane and Nucleoplasm -Liver	Lipids, Taurocholate	Macrophage lipid homeostasis, Placental lipid metabolism, Multidrug resistance (MDR)	(Kaminski et al., 2001; Dlugosz and Janecka, 2016; Imperio et al., 2019)
ABCA7	-Plasmatic membranes, endoplasmic reticulum, and Golgi apparatus -Bone marrow, spleen, brain, colon, liver, pancreas, lung, BBB (Blood Brain Barrier)	Cholesterol and Lipids, Beta-amyloid peptide, Ceramide	Phagocytosis of macrophage, Efflux of cholesterols and maintenance of lipids homeostasis in immune cells, Phagocytic removal of Beta-amyloid within the brain, Cancer overexpression in breast, cervical, lung, colorectal, and pancreatic.	(Fu et al., 2016; Liu et al., 2018; Zappe et al., 2023)
ABCA8	-Plasma membrane -Ovary	Cholesterol, Hydrophobic drugs	Cholesterol efflux, Regulation of sphingomyelin production in oligodendrocytes, Transport certain lipophilic drugs	(Tachikawa et al., 2018)
ABCA9	-Plasma membrane -Heart and adipose tissue	Monocyte, Macrophage	Monocyte differentiation and macrophage lipid homeostasis, Lipid transport	(Li et al., 2013; Chu et al., 2022)
ABCA10	-Plasma membrane -Muscle and heart	Cholesterol and lipids	Cholesterol responsive gene, Macrophage lipid homeostasis, Prognostic factor for BC	(Akiyama, 2014; Sheth et al., 2018)
ABCA12	-Plasma membrane, Cytosol, Lysosome -Keratinocytes, stomach	Cholesterol and lipids	Lipid homeostasis in the skin, prenatal diagnosis (Harlequin ichthyosis)	(Tomioka et al., 2012; Iritani et al., 2018)
ABCA13	-Plasma membrane -Bone marrow	Unknown	Lipid transport, hematopoiesis, Neurodevelopment within CNS (Central Nervous System), Bipolar disorder	(Tachikawa et al., 2018)

The mRNA gene expression data reveals that the expression level of the cell membrane transporter family demonstrated an increase in AMJ13 and MCF-7 cells. The greatest increases in expression were detected for *ABCA1*, which increased by 5.47 and 8.59 fold changes, respectively, while *ABCA3* showed 1.65 and 2.97 fold changes, respectively, as shown in Figure 2, revealing an overexpression pattern as a result of the interference with cisplatin and, consequently, the emergence of drug resistance.

Discussion

Even at the highest concentration of 1,000 µg/ml, only 49% to 60% of cancer cells were destroyed in the two types of cell lines, and this didn't show the expected cytotoxicity for such a high dose, which can prove that these cells appear to have developed chemoresistance.

Table 2. The Thermal Cycler Program.

No.	Steps	℃	Minute: Second	Cycle
1	RT. Enzyme Activation cDNA Synthesis	37	15:00	1
2	Initiation of the Denaturation	95	10:00	
3	Denaturation	95	0:20	40
4	Annealing	60,62	00: 20 Acquisition of Green	
5	Extension	72	0:20	

Due to the fact that the major of these chemosensitizers consider substrates for ABC transporters and therefore competed with the chemotherapeutics, high doses are needed to reach a sufficient amount of chemotherapy within cells of cancer (Ambudkar et al., 1999; Omran et al., 2017; Hussain et al., 2022). However, the doses used are very high for cisplatin which is usually effective at doses of 1 µg/ml in sensitive cell lines. While in the current cell lines, we used overdoses up to 1000µg/ml to explore the IC_{50} dose which is again considered very high compared to sensitive cell lines. It is found by other group of researchers that MCF-7 IC_{50} dose is 1.9µg/ml and the chemoresistance MCF-7 IC_{50} dose is 8.4µg/ml while in our current study found that it is about 90.23µg/ml which confirm the resistance of the MCF-7 cells used in our study which is about 90 times higher (Kashkoulinejad-Kouhi et al., 2021).

Morphological analysis confirmed the results of the cytotoxicity study, which shows that MCF-7 and AMJ13 cells at IC_{50} dose have presence of cell detachment and large viable cells remaining with less number of cells showing mild lesions such as cell rounding and nuclear condensation. The high dose of 202.2 and 90.23 µg/ml respectively, which is the IC_{50} values confirmed the chemoresistant nature of the cells. These results were also confirmed by Puspita and Bedford (2017), which described the chemoresistant cells as large cells with cytoplasmic granulation.

In our results, the amplification of the expression levels of the *ABCA1* and *ABCA3* genes related to the AMJ13, and MFC-7 cell lines revealing an overexpression pattern as a result of the interference with cisplatin and, consequently, the emergence of drug resistance. *ABCA1* protein has an important function in the regulation of the intracellular metabolism for cholesterol by mediating transmembrane transportation of phospholipids and intracellular unbound cholesterols to the Apo A-I (apolipoprotein A-I). Studies revealed that the upregulation of the *ABCA1* gene in H1299 non-SCLC cells resulted in an increased ability to resist chemotherapy (Islam et al., 2018; Min and Lee, 2021).

The presence of these transporters with excessive expression in cancer cells leads to the efflux of several therapeutic agents, resulting in their diminished efficacy in treating cancer. This phenomenon is evident in the emergence of MDR, as seen with cisplatin, doxorubicin, curcumin, and nitidine during the treatment of lung and breast cancer.(Sun et al., 2015; Hou et al., 2017). By reversing the inhibition of ABCA transporter expression, it has been observed an important function for ABC transporters within cancer stem cells. (Chen et al., 2017). Through histone deacetylase-2, the valproic acid decreases *ABCA1* expression and makes Non-SLCCs more sensitive to the chemotherapy drugs cisplatin. This could be just one of the several strategies for overcoming the drug resistance produced by elevated *ABCA1* expression (Ma et al., 2015; Baraa et al., 2016). Elevated expression with the ABCA gene can lead to an increase in the incidence of drug resistance and poorer outcomes for patients with a variety of cancers. The available data is heterogeneous and changeable among tumor types, revealing contentious and complex interactions that may be affected by the dissimilar biological background of the cellular context, which further complicates the whole situation (Pasello et al., 2020).

However, at the experimental level, the way these ABCA transporters work physiologically has different effects on cancer activity, and their relationships with tumor malignancy are more complicated than what has been documented for conventional members of ABC transporters, which act as drug efflux to mediate MDR. In particular, the expression of *ABCA3* represents a distinguishing feature of a certain subset of cancer stem cells that are highly resistant to chemotherapeutic drugs, and *ABCA5* has been linked to tumor stemness and the potential to metastasize in osteosarcoma (Saini et al., 2012; Naghibi et al., 2023). Furthermore, as stated by (Pasello et al., 2020), it is not feasible to determine the general relationship between *ABCA1* expression and tumor aggressiveness because of the divergent nature of this relationship, which can be either positive or negative, based on the type of cancerous neoplasm. Multiple studies provide support for the carcinogenic function of *ABCA1*. For example, (Aguirre-Portolés et al., 2018), It was founded that the upregulation of *ABCA1* with colorectal cancer is associated with the induction of the transformation from epithelial to mesenchymal and enhanced cellular invasion, thus promoting the stabilization protein Cavoelin1, while overexpression of *ABCA1* in prostate and ovarian cancers resulted in enhanced growth capacity and migratory potential. A similar upregulation of the ABCA transporter gene result was obtained by (Torres-Adorno et al., 2019), showing that upregulation of *ABCA1* effectively suppressed the polarization of the cell membrane induced by cisplatin and doxorubicin therapy, therefore blocking tumor cell death in triple-negative breast cancer (Makuch-Kocka et al., 2023). On the other hand, contrary evidence revealed that *ABCA1* has the opposite function in tumor suppression. Previous research has presented empirical data showing that the overexpression of *ABCA1* exerts an inhibited effect on tumor development in xenograft models of cell lines derived from patient malignancies (Smith and Land, 2012). In addition, (Moon et al., 2019), provided a description of the inhibitory effect of P53 on the mevalonate pathway. The cholesterols and non-sterol isoprenoids are both biosynthesized via this path, and it is extremely significant in a wide range of carcinogenic processes, that occurs through the transcriptional activation of the *ABCA1* gene. Comparable when P53 protein is absent, the removal of *ABCA1* promotes hepatic tumorigenesis in mice, confirming its role in tumor suppression (Mullen et al., 2016). It is also found in Iraqi patients that overexpression of excision repair cross-complementing 1 (ercc1) is associated with platinum drug resistance in breast cancer patients (Karim, 2017).

At the clinical level, BC has been observed to have significant upregulation levels of *ABCA2*, A3, A7, A12, and A13 compared with normal tissues. Conversely, *ABCA5*, *A6, A8, A9*, and *A10* exhibited substantially lower regulation (Hlaváč et al., 2013; Dvorak et al.,

2017). However, A reduction in *ABCA3* expression has been found to increase the possibility of tumor recurrence independently (Schimanski et al., 2009). Compared to tumors with a complete clinical response, patients with BC whose tumors showed an inadequate clinical response to neoadjuvant treatment and residual illness had significantly higher expression of *ABCA1* and *ABCA12* (Park et al., 2006; Group, 2015).

In conclusions, the present study provides evidence of the up-regulation of the *ABCA1* and *ABCA3* proteins in AMJ13 and MCF-7 cell lines and their association with the development of resistance to cisplatin in these cells. These results indicate that targeting the *ABCA1* and *ABCA3* genes is a potential strategy for reversing resistance to cisplatin in patients diagnosed with BC.

Author Contribution Statement

All authors contributed equally in this study.

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General

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Approval

If it was approved by Institute of Genetic Engineering and Biotechnology for Postgraduate Studies, University of Baghdad, Baghdad, Iraq, number 2860 date: 17/12/2019.

Ethical Declaration

The work approved by Institute of Genetic Engineering and Biotechnology for Postgraduate Studies, University of Baghdad, Baghdad, Iraq, number 2860 date: 17/12/2019.

Conflict of Interest

The authors declare that they have no conflict of interest.

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